REMARKS/ARGUMENTS

After entry of this amendment, claims 71-83 are pending in this application. New claims 71-82 are based on cancelled claims 40-50 and 55, respectively. New claim 83 finds support in the specification at page 13, line 5-7. The claim amendments introduce no new matter.

Applicants note with appreciation the courtesies extended by the Examiner in the interview on March 2, 2005. During the interview, the rejections under 35 U.S.C. § 112, first paragraph, were discussed as set forth in more detail below.

Rejection und 35 U.S.C. § 112, first paragraph

In the office action, the Examiner rejects the claims because the application allegedly fails to comply with the written description requirement. The Examiner asserts that in the absence of teaching as to the correlation between the enzyme substrates and the structure to be made, the claims lack written description. As has been explained in previous responses and during the telephone interview, the claims are not directed to the production of ***any*** glycoside product using any mutated glycosidase and any randomly selected donor and acceptor molecule. Instead, the invention is based, at least in part, on the discovery that a glycoside product that is the substrate of a particular glycosidase can be synthesized by the mutant form of that enzyme (a glycosynthase). To further emphasize this aspect of the invention, claim 71, which corresponds to original claim 40, specifically recites that the desired glycoside product is selected to be a substrate for a glycosidase enzyme having two catalytically active amino acids with carboxylic acid side chains within the active site.[1] Support for this claim language is replete throughout the application and is found for example, at page 7, first full paragraph.

Thus, as suggested by the Examiner, the current language specifically sets out the relationship between the product (a substrate of a glycosidase), the enzyme (a mutant form of that glycosidase) and the donor and acceptor molecules (based on the products of the

[1] One of skill will recognize, however, that the glycosidase need not be a naturally occurring enzyme. Thus, for example, a glycosynthase of the invention could catalyze the synthesis of a product that is not recognized by a naturally occurring glycosidase.

glycosidase-catalyzed reaction). Those of skill will appreciate that, like any enzyme-catalyzed reaction, the selection of a desired final product will depend upon the identification of a glycosidase that recognizes it as a substrate. Once the final product and desired enzyme are identified, the selection of donor and acceptor molecule is well within the skill in the art.

The Examiner continues to express concern that the claims encompass a "highly variant" genus of enzymes, donors and acceptors. In this regard, the Examiner asserts that "representative species" within each of these genera must be disclosed. As described above, the amended claims now clarify that the desired product of the claimed methods is determined by selection of a glycosidase that recognizes the product as a substrate. Once the enzyme and product are selected, the donor and acceptor molecules are largely determined. Thus, applicants understand this aspect of the rejection to be essentially a question of whether the specification provides adequate description of the genus of glycosynthases used in the methods of the invention.

As an initial matter, applicants respectfully disagree with the Examiner's statement that only a single example is provided in the specification (the AbgE358A mutant). The specification also discloses two other mutant glycosidases. The first is a mutant α-amylase (human or porcine) with the aspartic acid at position 197 replaced with another amino acid such as, for example, alanine. While the second is a yeast α-glucosidase with the aspartic acid at position 216 replaced. These two enzymes are specifically described at page 11, lines 3-7.

With regard to description of the genus of enzymes, it is well settled that a representative number of species to support a genus does not require actual reduction to practice. *See* MPEP § 2163 II(A)(3)(a) ("[a]n adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention"). In the present case, the genus of enzymes used in the invention is defined by the mutation to certain key residues in the catalytic site. In particular, catalytically active residues with carboxylic acid side chains are replaced with residues having a non-carboxylic acid side chain.

In the response mailed February 21, 2003, applicants provided extensive evidence with regard to the level of knowledge of glycosidase enzymes at the time of the invention. As

explained there, the amino acid sequence and crystal structures of a number of glycosidase enzymes were known.[2] Also, carboxylate-containing amino acid residues recognized as within the active site of many of the glycosidase enzymes of diverse classification had been designated.[3] Still further, amino acids within the active site of many glycosidase enzymes had been identified by various other means, including the use of modified substrates that can form a relatively stable glycosyl-enzyme intermediate, *e.g.*, a 2-deoxy-2-halo glycosyl derivative (*see* page 9, lines 6-12 of the specification and Sinnott, *Chem. Rev.* 90:1171-1202 (1990)). Although glycosidase enzymes were recognized in the art at the time of the present invention as being diverse in primary amino acid sequence and overall structure, the enzymes were known to share many structural and functional features in their active sites.[4] This information was also available at the time of the invention on a web site: http://afmb.cnrs-mrs.fr/CAZY/index.html

In light of the above, it is clear that the structure and function of a broad range of glycosidases were known at the time of the invention. Thus, the present description provides sufficient, relevant, identifying characteristics of the genus of glycosidases useful in the

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[2] *See generally , e.g.*, Henrissat and Bairoch, *Biochem. J.* 316:695-696 (1996) (citing pre-filing date references). *See also, e.g.*, Janecek, *FEBS Lett.* 377:6-8 (1995) (describing amino acid sequence comparisons of $(\alpha/\beta)_8$-barrel glycosyl hydrolases); Jenkins *et al.*, *FEBS Lett.* 362:281-85 (1995) (comparison of crystal structures of (α/β)8-barrel glycosidases); Hengstenberg *et al.*, *FEMS Micrbiol. Rev.* 12:149-63 (1993) (reviewing 6-phospho-β-galactosidases, noting sequence similarities with other glycosidases and crystallisation of the staphylococcal and lactococcal enzymes) (All submitted February 21, 2003).

[3] *See, e.g.*, Barrett *et. al.*, *Structure* 3:951-60 (1995) (pp. 953-955); Jenkins *et al.* at 282; Hengstenberg *et al.* at 159-160; Voorhorst *et al.*, *J. Bacteriol.* 177:7105-11 (1995) (pages 7107-09); Janecek at 6 (Abstract and second column, noting positioning of "well-known catalytic aspartate" in $(\alpha/\beta)_8$-barrel glycosyl hydrolases); Leah *et al.*, *J. Biol. Chem.* 270:15789-15797 (1995) (page 15793, Fig. 5) (All submitted February 21, 2003).

[4] *See, e.g.*, Henrissat *et al.*, *Proc. Natl. Acad. Sci. USA* 92:7090-7094 (1995); Jenkins *et al.*; Janecek; Mackay *et al.*, *Biosystems* 18:279-92 (1985) (noting that diversity of glucanases allows identification of "their most highly conserved (and presumably functionally important) regions" (Abstract) and showing entire conservation of certain residues by sequence alignment (Fig. 2)). *See also, e.g.,* Legler, *Adv. Carb. Chem. Biochem.* 48:319-84 (1990) (reviewing inhibition data showing diverse glycosidase enzymes to effect catalysis by the same basic mechanism) (All submitted February 21, 2003).

invention so that a person skilled in the art would recognize that the present inventors had possession of the claimed invention. Withdrawal of the rejection is respectfully requested.

The claims also stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement.

During the interview, clarification of the nature of the leaving group attached to the donor substrate was discussed. Applicants understand in light of the interview that the Examiner accepts the points made in Dr. Withers' Declaration that one of skill could readily identify leaving groups that would be useful in the claimed methods. Applicants note that in the Office Action, the Examiner appears to accept that a claim directed to donor molecules comprising small leaving groups would be enabled. Claim 71 includes an explicit limitation that the donor molecule comprises a good leaving group. Support for the claim language is found for example at page 12, lines 4-7. In light of the evidence of record on this point, Applicants believe this limitation should address the Examiner's concerns with regard to the nature of the leaving group on the donor molecule.

In the Office Action and during the interview, the Examiner acknowledged that the specification is enabling for the use of a mutant of a retaining glycosidase wherein the catalytic nucleophilic amino acids are replaced with non-nucleophilic residues to form an oligosaccharide. Thus, the Examiner apparently acknowledges that once an enzyme and product are identified, the selection of an appropriate donor and acceptor is well within the skill of the art. Applicants believe the rejection of claim 72 is improper since this claim is directed to the subject matter that is acknowledged to be enabled by the Examiner.

In the rejection, the Examiner goes on to express concern about the large number of different glycosidases having different structures, functions and substrate specificities. Since the Examiner has acknowledged that methods are enabled for retaining glycosidases, applicants understand this rejection to be concerned primarily with mutants of inverting glycosidases. As explained in detail above, the structure of glycosidases was well known at the time of the invention. In addition, since the pending claims specifically recite the selection of the product based on the substrate of the glycosidase, the enablement issue should focus on whether one of skill could prepare a glycosynthase from a specific glycosidase using the teachings of the present

application. As explained below, the skilled artisan could readily identify the appropriate residues to modify for any particular enzyme, without undue experimentation.

As noted previously, in addition to the vast literature on the structure and function of glycosidases, the specification specifically describes known methods for identifying the catalytic amino acids. *See, e.g.*, specification at pages 9-11. These methods include, *e.g.*, the use of modified substrates that can form a relatively stable glycosyl-enzyme intermediates; inspection of three-dimensional structure through X-ray crystallography and NMR spectroscopy; and site-directed mutagenesis coupled with determination of the glycosyl fluoride substrate utilized by the mutant enzymes. As explained in detail in the response mailed Feburary 21, 2003, many of these methods for identifying the catalytically active carboxylates were generally known to the skilled artisan as of the effective filing date. *See, e.g.*, Miao *et al.* (use of modified substrate to form glycosyl-enzyme intermediate); Jenkins *et al.* (use of crystal structure and sequence data to determine general position of catalytic carboxylates).

In addition, the inventor's Declaration submitted June 3, 2002, demonstrates that different mutant glycosidases as described in the specification yield the requisite glycosynthase activity, regardless of the "rest of the enzyme structure." As explained previously, this declaration provided evidence of the level of skill in the art at the time of filing and that the disclosed mutated glycosidase would have been operative as described. That Declaration, provided evidence, for example, that the catalytically active glutamic acid residue at amino acid 537 of *E. coli* Lac Z β-galactosidase could be replaced with serine, a smaller amino acid lacking a carboxylic acid side chain.

In addition, the Declaration provided evidence from a number of publications demonstrating that applying the teachings of the present application, those of skill could make and use mutatnt glycosisases within the scope of the invention. Each of these references describes a mutant glycosidase, in which an active site carboxylic acid-containing amino acid is converted to an amino acid which does not contain a carboxylic acid side chain.

Nashiru *et al.*, *Angew Chem. Int. Ed.* 40:417-420 (2001) disclose a β-glycosynthase derived from a mannosidase cloned from *Cellulomonas fimi* (one of the types of glycosidases listed on page 7 of the application), in which the active site nucleophile Glu519 was

converted to either alanine or serine. Both provided an enzyme that was inactive as a hydrolase but retained mannosynthase activity. The active site nucleophile was identified by trapping the covalent mannosyl-enzyme intermediate with 2-deoxy-2-fluoro β-D-mannosyl fluoride, followed by proteolysis and sequencing of the labeled peptide (a method described in the specification).

Trincone *et al.*, *Bioorganic & Medicinal Chem. Lett.* 10:365-368 (2000) disclose a glycosynthase, derived from the β-glycosidase of *Sulfolobus solfataricus*, in which that active site glutamic acid residue (Glu387) of the wild-type enzyme is mutated to either alanine or glycine. The nucleophile had been previously identified as Glu387 using various methods, such as use of a conduritol B epoxide inactivator (a method known in the art as of the effective filing date).

Mayer *et al.*, *FEBS Lett.* 466:40-44 (2000) describes a Glu358Ser mutant of *Agrobacterium* β-glycosidase (a different mutant of the enzyme in the specific examples of the application replacing the carboxylic acid-containing amino acid (Glu) with a non-carboxylic acid-containing amino acid that has a side chain of approximately equal or smaller size) which has improved glycosynthase activity resulting in higher yields, reduced reaction times, and enhanced synthetic repertoire.

Mayer *et al.*, *Chem. & Biol.* 8:437-443 (2001) describes mutants Glu358Cys and Glu358Gly of *Agrobacterium* β-glycosidase (also different mutants of the enzyme in the specific examples of the application replacing the glutamic acid with additional amino acids having a side chain of approximately equal or smaller size) and shows them to have no hydrolysis activity and different rates of glycosynthase activity.

Fort *et al.*, *J. Amer. Chem. Soc.* 122:5429-5437 (2000) describes a glycosynthase prepared by replacing the catalytic nucleophile Glu197 in endonuclease Cel7B from *Humicola insolens* with alanine. Glu 197 had been identified as the catalytic nucleophile by trapping of the covalent glycosyl-enzyme intermediate and comparison of amino acid sequence with other related glycosidase family members (methods described in the specification).

Malet *et al.*, *FEBS Lett.* 440:208-212 (1998) describes mutant forms of glucanases from *Bacillus licheniformis*. The paper notes that two glutamic acid residues Glu138 and

Glu134 had been identified as the catalytic acid/base and the nucleophile, respectively, citing a pre-filing date reference (1994). The mutant form Glu134Ala was prepared and shown to lack hydrolysis activity and to act as a glycosynthase.

The mutations described above and the resulting inactivation of hydrolysis activity and conferring of glycosynthase activity are as described in the specification as filed. The sites for mutation of the wild-type enzyme either had been identified as of the effective filing date or were determined using methods described in the specification or known in the art. Applicants believe it is also apparent from these disclosures that the time and effort to produce the glycosynthases described in the references was not more than would be expected using the knowledge and skill in the art at the time of filing and the methods described in the specification. For these reasons, Applicants believe that the post-filing references are admissible as additional evidence of enablement. In light of the remarks above, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

Rejection under 35 U.S.C. § 112, second paragraph

The rejection of claims 40-50 and 55 for allegedly being indefinite is rendered moot by cancellation of these claims. New claims 71-82 do not contain the allegedly indefinite language.

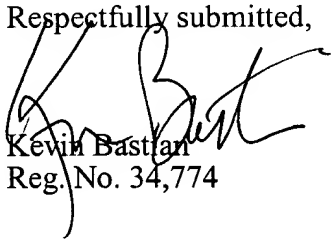
Double Patenting Rejections

Applicants acknowledge the rejection of the claims under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 5,716,812 and claims 1 and 2 of U.S. Patent No. 6,284,494. As set forth in Applicants prior responses, a terminal disclaimer will be submitted, if required, once an indication that the claims are otherwise in condition for allowance has been received.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,



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